ANALYSIS OF FACTORS ASSOCIATED LESS DIFFERENTIATED T CELLS IN BREAST CANCER FOR IMPROVEMENT OF ADOPTIVE T CELL THERAPY

Jeong-Han Seo, Hyeonjin Lee, Gyungyub Gong, Hee Jin Lee, Sung Wook Jung.

1 NeogenTC Corp, Seoul, South Korea, Republic of; 2 Asan Medical Center, Seoul, South Korea, Republic of; 3 University of Ulsan College of Medicine, Seoul, South Korea, Republic of

Background The survival and proliferative potential of T cells in vivo is related to the differentiation status of T cells. Less differentiated cells show prolonged survival duration and effective anti-tumor response for adoptive T cell therapy. To improve in vivo sustainability of adoptive T cell therapy, we analyzed factors associated with less differentiated T cells in breast cancer.

Methods We performed a single cell RNA (scRNA) sequence of CD45+ immune cells from 21 breast cancers. Basic analysis was performed using the Seurat package, and immune cells were clustered through UMAP. CD8+ cluster was defined using CD3E, CD8A, and CD8B markers. Differentially expressed genes between differentiated and less differentiated cells were assessed in 3 conditions [SELL−CCR7− (Tem) vs. SELL+CCR7+ (early memory T cell); CD39+CD69+ (terminally differentiated T cell) vs. CD39−CD69− (Tscm phenotype); KLRG1+CD127− (terminal effector cells) vs. KLRG1−CD127+ (pre-memory cell phenotype)]. Genes related to transcription factor and metabolic genes and epigenetic pathways were identified. We also derived data from 5 breast cancer scRNA datasets identified in the NCBI-Gene Expression omnibus and performed the same process as above.

Results 29,102 CD8+ T cells from 6 datasets were analyzed (GSE141665, n=1,767; GSE114724, n=8,400; GSE161529, n=3,023; GSE176078, n=8,036; GSE110686, n=1,214; in house, n=6,662). Based on the 3 conditions in each dataset, up-regulated genes were classified by comparing more and less differentiated phenotypes, and the identified genes were annotated as transcription factors, metabolic genes, and epigenetic genes (transcription factors, n=138, 128, 246, 33, and 546; metabolic genes, n=324, 230, 700, 1,224, 63, and 1,262; epigenetic genes, n=24, 34, 53, 106, 7, and 546 for each dataset, respectively). Total 486 genes, which were up-regulated in less differentiated CD8+ T cells, were obtained by sorting two or more overlapping conditions among the 3 conditions in each dataset (GSE141665, n=141; GSE114724, n=154; GSE161529, n=305; GSE176078, n=840; GSE110686, n=22; in house, n=974). Among 486 genes obtained from 6 datasets, 17 genes including FOS, JUNB, and LEF1 were present in all datasets.

Conclusions By combining 21 tumor samples and 5 public datasets, we identified 17 up-regulated genes, which are transcription factors or genes associated with metabolic and epigenetic pathways, in less differentiated CD8+ T cells. Further studies modulating these genes may suggest the possibility of returning terminally differentiated T cells to less differentiated status to improve the T cell therapy.

Ethics Approval The study was approved by the Institutional Review Board of Asan Medical Center, approval number IRB 2016-0935.